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Fatty Acids in an Irradiated Chicken Product.

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FATTY ACIDS IN AN IRRADIATED CHICKEN PRODUCT

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ABSTRACT

Lipid studies were conducted on samples of canned non-irradiated and irradiated chicken-based pet food products. The fatty acids of these samples were isolated and identified using gas-liquid chromatography. Classes of lipids in the extracts of non-irradiated and irradiated samples were separated and tentatively characterized by thin-layer chromatography. Total extractable lipid was determined gravimetrically.

Phospholipids, sterols, free fatty acids, triglycerides and sterol esters were demonstrated on thin-layer plates in the samples of both non-irradiated and irradiated chicken products. The triglyceride class was observed to be the most abundant fraction of the total lipids in both types of samples.

Fatty acids tentatively identified by gas-liquid chromatography in both the non-irradiated and the irradiated samples were caprylic acid (C_8), capric acid (C_{10}), undecylenic acid ($C_{11=}$), myristic acid (C_{14}), tetradecenoic acid ($C_{14=}$), palmitic acid (C_{16}), palmitoleic acid ($C_{16=}$), stearic acid (C_{18}), oleic acid ($C_{18=}$), linoleic acid ($C_{18=}$), and arachidic acid (C_{20}). The fatty acids that constituted 79.97 per cent of the total fatty acids in the irradiated samples and 79.07 per cent of the total fatty acids in the non-irradiated samples were identified as palmitic acid, oleic acid and linoleic acid. There were no differences detected between

relative fatty acid composition of the total lipid extract of non-irradiated and irradiated samples.

In regard to the major fatty acids identified on gas liquid chromatograms, the composition of the total extract and the lipid fractions in both the non-irradiated and irradiated samples were similar. The relative fatty acid composition of the triglyceride fraction was the closest to the total extract. There were no differences in the relative composition of the fatty acids of the triglyceride fractions of the non-irradiated and irradiated samples.

INTRODUCTION

Research has been reported in several countries on the application of radiation energy for food preservation and processing with the object of contributing to the world's food supplies by increasing its storage stability. Irradiation of food as a means of preservation is the first truly new food preservation method in the last hundred years. Because this is a new method, the government regulatory agencies need sufficient evidence that the foods processed by irradiation are safe and nutritionally adequate.

Radiation sterilization of chicken products would be extremely beneficial because of the high incidence of Salmonellae in chicken. The importance of destroying Salmonellae in eggs, poultry, and animal feeds as well was felt to be so serious that a meeting on the control of this microorganism by irradiation was held in 1962 under the sponsorship of the International Atomic Energy Agency (1963). The issue was thought to be even more significant in improving the public health status of the world than as an important tool in food processing.

The acceptability of meats preserved by irradiation has been hampered by the formation of off-flavors and odors. Studies of the problem have been in progress in many laboratories for several years to discover the nature of irradiation flavor and how to prevent it. It has been proposed that this flavor and odor is caused by volatile

chemical compounds produced by radiation impact on the lipid molecules (Merritt, 1966).

The purpose of the present study was to determine if the fatty acids of a chicken-based pet food product would be altered and to what extent by Cobalt-60 irradiation at the presently acceptable sterilization level of 4.5 Mrad. Many investigations have been undertaken on the separation and identification of the by-products of irradiated fatty acids, alone or esterified in the various lipid classes (Lang and Bassler, 1966; Mead, 1952; Merritt et al., 1966). There has been very little published on the specific effect of irradiation directly on fatty acids irradiated in their natural state in meat products. This study was undertaken to provide information on the quality of the fatty acids after irradiation. The chicken-based pet food was a readily available chicken product for work on irradiation vs. non-irradiation of a food.

Contrary to past practices, today's family pet enjoys a convenience diet product bought at the local supermarket along with the regular family groceries. Projected sales in pet foods for 1968 were set at $900 million. Today's pet foods range from gourmet items such as burgundy beef in gravy, meat balls or chicken croquettes to nutritionally balanced canned and dry foods (Pinkos, 1968).

Improved technology in the preservation of foods has obvious implications for the food manufacturer. The importance of food preservation is particularily relevant in certain regions of the

world where up to thirty percent of the harvested foodstuffs are being lost because of damages by animal pests and microorganisms.

LITERATURE REVIEW

Literature on the effect of radiation sterilization on fatty acids in meats and poultry is rare. However, studies on the flavor and odor of irradiated meats have been extensively reported. Experiments on both meats and poultry are reported in this literature review.

The basic requirements for sterile products using nuclear energy are reasonably well established and were determined by Urbain (1966). The requirements include the following: An amount of absorbed radiation (dose) sufficient to destroy all spoilage microorganisms present. Of those involved, the most radiation resistant is Clostridium botulinum. The dose presently considered necessary for meats and poultry having no added salt or acid is 4.5 Mrad (million rads) when the irradiation is carried out at or above normal refrigeration temperatures. This dose destroys all spoilage organisms, including C. botulinum. The product should be in a closed container in order to prevent recontamination. Naturally occurring enzymes should be inactivated. Since the dose for microbial sterility does not accomplish this, a supplemental treatment is needed. Presently the only effective method available is to heat the food to approximately 70°C. Ingram and Roberts (1966) reported similar requirements for obtaining sterile products using radiation. A radiation dose approaching 5 Mrad were required to inactivate the spores of C.

botulinum. Since the spores of C. botulinum are most resistant to radiation, it has been understood that the first requirement of any process of sterilization must be to control this microbial species. The current concept of radiation sterilization is based on the assumption that the required dose to kill C. botulinum needs to be comparable in effectiveness to that attained by heat processing methods. Computation using the decimal reduction dose system is commonly employed in the canning industry. Expressing the decimal reduction dose on a linear plot, the ordinate is the logarithm of the surviving fraction of C. botulinum and the abcissa is the irradiation dose. When the survival curve transverses one log cycle, the bacterial population will be mathematically reduced by a factor of 10. For example, if the original number of microorganisms was 10^{12} before radiation, it would require a total of 12 decimal doses to reduce the population to 1.0. Since each decimal dose is that segment of radiation that will decrease the count by 90 percent, an accumulation of 13 decimal doses would be necessary to have less than one survivor remaining, or in other words, produce a sterile product. According to Ingram and Roberts (1966), some bacteria were known to survive irradiation doses of 5 Mrad, e.g. Micrococcus radiodurans. The radiation resistant microorganisms were killed in the heat treatment which was intended to prevent enzymatic degradation in foods for long storage. These microorganisms were heat sensitive and thought to be harmless. Hansen et al. (1963) found that pre-irradiation heating of the chicken to approximately 80°C markedly inhibited development of an objectionable red color. This color

development was noticed in radiation sterilized chicken stored anaerobically at elevated temperatures in the absence of oxygen. This observation was confirmed as an essential step in radiation preservation by other workers (Cain et al., 1958; Coleby et al., 1961; Hannan and Shepherd, 1959).

Urbain (1966) reported that large piece meats and poultry when thermally processed to sterility were overcooked. Whole chicken and hams suffered severe texture damage, overtenderness, and had an off-flavor, therefore the process is not commercially feasible. Organoleptic data indicated that radiation sterilized chicken scored the same rating as the counterpart chicken products preserved by freezing. The irradiated meats, pork, beef roast and bacon had acceptance equal to or close to the frozen counterpart. It was observed in this study that thermally processed meats did not receive the same acceptance. Hanson et al. (1964) found that deep-fat frying of chicken which had been irradiated at 4.5-4.6 Mrad while nitrogen packed and stored at 21°C and 38°C reduced unpleasant odor and flavor. This was an improvement over the cooking methods without fat. Hanson et al. (1964) also observed that there was little irradiation odor and flavor formed when samples were irradiated at -20°C or lower from taste panel results. Heighiman (1965) reported that enzyme inactivated and then radiation sterilized chicken stored for 20 months at 70°F received the same organoleptic score as the fresh chicken did at the beginning of the storage period. Panelists scored the irradiated

chicken as acceptable and stable after storage for 21 months at 21°C and 18 months at 38°C.

Gernon and Seaton (1962) reported results of investigations on chicken thighs. The chicken had been irradiated at 4.5 Mrad and stored at 22°C. A panel of 10 judges rated the product on a 9 point Hedonic scale. Scores of 7.1, 6.7, 6.8, and 6.9 after 1, 4, 9, and 18 months of storage were reported.

Hannon (1959) found that irradiation odor decreased during storage of raw chicken irradiated at 2.0, 3.5, and 5.0 Mrad. The flavor of the chicken deteriorated during storage at 25 C and 37 C probably due to enzymatic changes. This was verified by the work of Hanson et al. (1964). He studied chicken irradiated at a dose of 4.6 Mrad and stored for 1, 3, and 6 months. The odor and flavor were less intense in irradiated samples stored at 21°C and 38°C than in irradiated controls held at -34°C. These samples were heated to 68°C and held there for 45 minutes prior to nitrogen packing with irradiation at ambient temperature. Further study by these investigators revealed that formation of irradiation odor and flavor were significantly inhibited when irradiation was carried out at -20°C. Irradiation at -6°C or -10°C did not produce this inhibitory effect. Coleby et al. (1961) irradiated beef and pork at lowered temperatures. They found little effect on odor and flavor when irradiation took place between 0°C and 20°C. Rapidly increasing protection was afforded the beef and pork samples when irradiation took place between freezing and -20°C

and only slightly greater influence at still lower temperatures down to -196°C. Coleby et al.(1961) hypothesized that an important part of the protection was due to freezing of the free water in the tissue. By cooling the samples to the desired temperatures, protection was never apparent until the temperature was less than -3°C, while if the meat was first cooled to a lower temperature, then protection was observed even with samples irradiated at -1°C. They speculated that although crystallization of water may be responsible for some of the protection, freezing would also alter the rates of reaction of the free radicals formed by irradiation. The general course of subsequent reactions was thought to be affected by the rates of reaction of the free radicals. Microbiologically the protection of vegetative microorganisms by freezing was essentially the same as the protection of the quality on meat. It appeared that the increased dose of radiation required for microbial sterility might cancel the benefits due to irradiation while the samples are frozen. The observation that sterilization was done to kill spores and that the vegetative cells would be killed by the heat treatment prior to irradiation, and that spores were not generally protected by freezing was made by Coleby (1961). The gain due to freezing was expected to be considerable.

Ground beef irradiated at levels up to 7 Mrad was fed to dogs as 35 percent of the dry matter of their diets. No measurable influence on growth rate, reproductive performance or general health of either male or female beagles over a three year experimental period were recorded. No deviation from the normal in hematology, X-rays of the

bones, growth and reproduction were found in these dogs over a period of 120 weeks. Similar results were demonstrated by McCay and Rumsey (1960) in a feeding study in which chicken stew was fed to dogs as 35 percent of the dry matter content of the diets. Dixon et al.(1961) fed bacon which had been irradiated at 5.58 Mrad to mice over a two year period and found no major differences in these mice and the controls. No gross or histopathologic lesions, carcinogens or growth altering substances were reported in the study. Deichmann (1961) obtained similar results feeding dogs and rats a beef stew irradiated at levels of 2.79 and 5.58 Mrad for two years.

Lang and Bassler (1966) recorded a variety of biological functions in a long-term feeding experiment on rats fed with oils which had received different degrees of irradiation. Soybean oil was fed to the rats in these experiments. Soybean oil is rich in unsaturated fatty acids which were considered to be highly susceptible to irradiation. The oil was irradiated at doses of 2, 5, 10, 50, and 100 Mrad. In Table 1, taken from the work of Lang and Bassler (1966), are listed the fatty acid composition of the irradiated soybean oils. There was no tendency toward decrease of the dienoic and trienoic acids due to irradiation up to 50 Mrad. In another experiment by the authors irradiation with 100 Mrad caused a definite decrease in dienoic and trienoic acids, the former decreasing from 49 percent to 20 percent and the trienoic acids decreased from 5.5 percent to 1.5 percent. Feeding rats the oil irradiated at 100 Mrad caused immediate adverse effects. Growth retardation, decreased food

Table 1. The fatty acids of irradiated soybean oil (Lang and Bassler, 1966).

Radiation Dose	Fatty Acids				
	Saturated		Monoenoic	Dienoic	Trienoic
	C_{16}	C_{18}	C_{18}	C_{18}	C_{18}
(Mrad)	- - - - - - - - - - Percent - - - - - - - - - -				
0	11	3.6	24	49	6.2
2.5	10	3.4	21	50	7.7
10	11	3.8	22	49	6.7
50	11	4.0	21	45	8.4

efficiency, and an increase in mortality were recorded for those rats fed the oil irradiated at 100 Mrad. After 6 months, 75 percent of the animals which had been fed the oil irradiated at 100 Mrad had died. For 24 weeks growth, food efficiency and reproduction were normal in rats fed the oil irradiated at 50 Mrad. Increased mortality was noticed after 40 weeks when the rats were fed a diet of oil irradiated at 10 Mrad. The oil which had been irradiated at 2.5 Mrad and fed to the rats did not cause any adverse effects on the rats during the duration of the experiment which was 80 weeks. The cause of death of the rats that were fed the oil irradiated at 50 Mrad was not determined by Lang and Bassler (1966). They discarded the idea that peroxides were responsible for the toxic effects of irradiation. Their experiments showed that the peroxide content of irradiated soybean oils was rather low and no correlation was found between the toxicity and peroxide values. The authors suggested that the toxicity could be caused by the increase in dimers and polymers which they found to be the main chemical changes in soybeans after irradiation.

Hansen (1966) summed up the means of reducing undesirable flavor and odor changes due to irradiation. He suggested several procedures including dose modifying processes like the use of free radical acceptors, irradiation at low temperatures, irradiation at a very high dose rate, or the exlucion of oxygen during and after irradiation. The use of odor absorbants and dose reductions were also suggested. Dose reductions could be accomplished by combination treatments, irradiation at high temperatures, use of sensitizers,

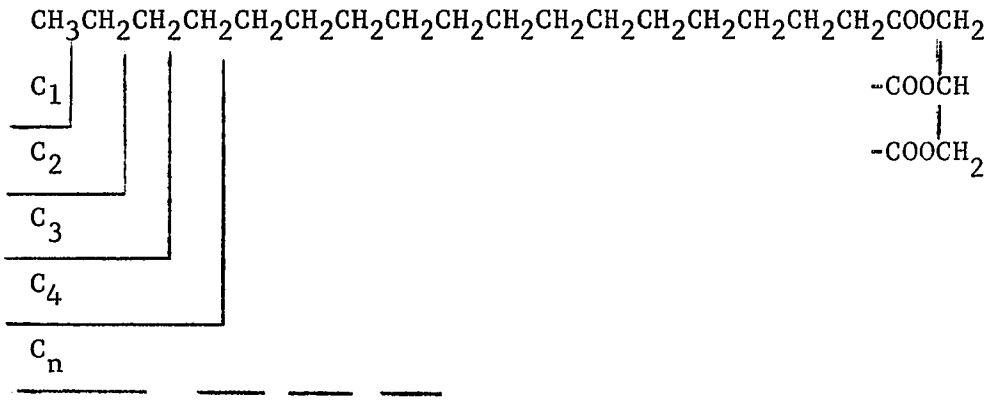
or the use of an uneven dose distribution. In conjunction with these suggestions, Hansen (1966) explained the mechanisms by which chemical changes can be brought about by ionizing radiations as follows: direct action, the molecule undergoing a change itself becomes ionized or excited by the passage through it of an electron or any other atomic particle; or indirect action, the molecule undergoing the changes does not absorb the energy but receives this by transfer from another molecule. In animal tissue which has a high water content, the main attack occurs by the indirect action, whereas the primary effect on bacteria is thought to occur by the direct action of ionizing rays. The free radical acceptors remove the free radicals before they can cause flavor changes. Attempts have been made to include a small package of activated charcoal in cans with irradiated meat to absorb the volatiles produced because of the great surface area of the charcoal. Hansen (1966) suggested that the most obvious method for obtaining sterility at a reduced dose was to combine irradiation with another bacteriocidal treatment such as heat, curing, antibiotics, etc. Hansen (1966b) reported that some combination treatments such as irradiation with heat, curing or antibiotics had been tried with success. He also reported that some interest had been shown in the use of sensitizing agents to decrease the microbial radioresistance. Hansen (1966) concluded that the formation of off-flavors in irradiated meat products was the most important obstacle to a successful application of irradiation in meat processing. He

felt it was essential that future research should concentrate on finding means to reduce the irradiation flavor. Yu et al. (1968) reported some success in reducing the Browning reaction caused by irradiation in seafood products by the use of a combination of antioxidants (10 percent butylated hydroxytoluene, 6 percent propyl gallate, 6 percent citric acid, and 12 percent propylene glycol) mixed with the food.

The use of more reliable and sensitive methods for the collection, separation and identification of volatile compounds has resulted in a greater knowledge of the effects of irradiation of foodstuffs. Progress in elucidating the chemistry of sensory changes observed in irradiated meats and meat fats has been directly related to the increased application of sophisticated analytical tools. The gas-liquid chromatograph and the mass spectrophotometer are examples of these tools. Early investigations into the chemical causes of the off odors and flavors implicated different constituents of meats as the precursors and different irradiation products as the cause (Batzer and Doty, 1955; Schultz et al. 1956; Cain et al., 1956; Mead, 1952; Pollister and Mead, 1954; Dugan and Landis, 1956; Slover and Dugan, 1957; Batzer et al., 1957; Artar et al., 1961; Thompson et al., 1961; Bautista et al., 1961). Some of the presumed irradiation products from meat proteins were amines and a product formed from the condensation of the sulfhydryl groups with carbonyl compounds. The lipid fraction was believed to produce peroxides, carbonyl compounds, and hydrocarbons by early workers who used chemical methods of analysis.

Merritt et al., (1959) were the first to demonstrate high vacuum-lower temperature distillation of the volatile of irradiated beef. This procedure was followed by subsequent gas chromatographic separation and mass spectrometric analysis. The meat had been irradiated at 6 Mrad using a Cobalt-60 source. Dimethyl disulfide and isobutyl mercaptan were identified and determined to be produced solely by the irradiation. After initial experiments, extensive reporting on the refinements of the analyses were made (Merritt, 1959; Merritt, 1960; Merritt and Walsh, 1963; Merritt et al., 1964; Merritt et al., 1965). A final review of this work was reported in a general report (Merritt et al., 1966). The discovery of the existence of hydrocarbons as the major components produced in irradiated meats was the most significant finding. A comprehensive analysis of the volatiles from irradiated ground pork, mutton, lamb, and veal as well as beef was carried out by Merritt (1966). All of the samples included in this work were irradiated under vacuum at a dose of 6 Mrad and complete analyses of the volatiles demonstrated the presence of more than 50 compounds. Alkanes, alkenes, alkynes, aromatic hydrocarbons, alcohols, ketones, aldehydes, esters, and sulfur compounds were identified in the irradiated samples. Merritt and his colleagues supported the hypothesis that radiation products were the result of direct bond cleavage. They used as an example a glycerol stearate molecule, below. Irradiation would cause scission of the bonds at all points of the chain. Recombination or hydrogen termination of

the resulting alkyl free radicals cause the formation of all the n-alkanes from methane to heptadecane.

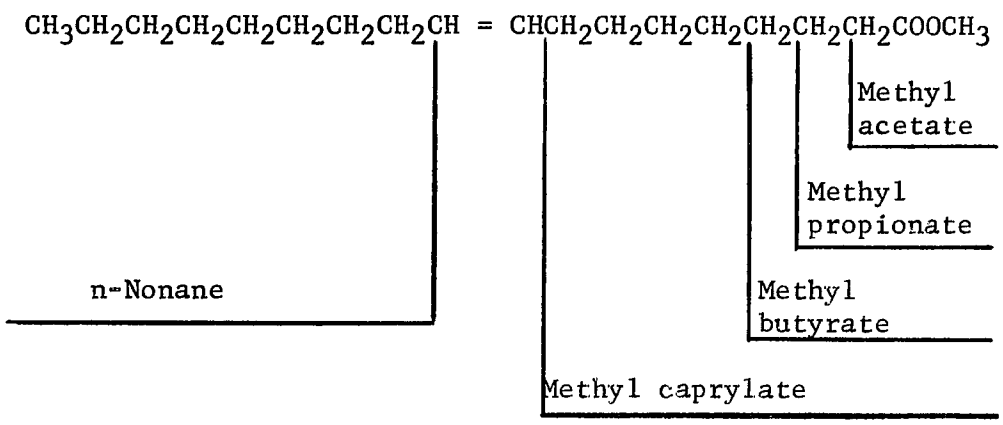


Alkanes to pentadecane were found in good yield from the irradiated meats. A homologous series of alkenes were detected in large quantity which indicated that secondary collisions occurred. The alkenes were formed by extraction of a second electron in the secondary collisions. These investigators found support for their hypothesis on fatty acid cleavage in a study of the radiation products induced by the irradiation of methyl oleate and methyl stearate. In Table 2 is shown the irradiation products which were formed when methyl stearate, methyl oleate or tristearin was irradiated by Merritt et al., (1966). The principal products to be alkanes, alkenes, and a homologous series of methyl esters. The highest member of the alkane series found in irradiated methyl oleate was n-nonane and the highest methyl ester was methyl caprylate. The series of unsaturated hydrocarbons went up to C_{10}. The authors felt that these compounds could arise from methyl oleate by the mechanism described for tristearin as follows:

Table 2. Radiation products formed on irradiation of three lipid compounds (Merritt et al, 1966).

Compound Irradiated	Type of Product Formed		
	n-alkanes	n-alkenes	methyl esters
Methyl stearate	C_1[a] — C_{13}	C_2 — C_9	C_2 — C_{10}
Methyl oleate	C_1 — C_9	C_2 — C_{10}	C_2 — C_9
Tristearin	C_1 — C_{13}	C_2 — C_{10}	

[a]Number of carbons in the product formed



Merritt and his colleagues felt that most of the other products found in irradiated meat volatiles may also have been accounted for by mechanisms associated with alkyl free radical formation in the fat.

Champagne and Nawar (1969) also observed that hydrocarbons were the major radiolytic products in fats. This was in agreement with Merritt (1966). The combination gas chromatography-mass spectrometer system was also used by these investigators to separate and identify the volatiles formed in beef and pork fats by irradiation. Some additional irradiation products were reported for the first time in this study (Champagne and Nawar, 1969). They were alkadienes and some of the longer chain alkanes and alkenes. These workers not only separated and identified the irradiation products but they also did a quantitative study of the irradiation produced volatiles. The major amount of hydrocarbons produced possessed either one or two carbon atoms less than the major fatty acids present in the fats studied. Champagne and Nawar (1966) felt that radiolytic splitting of fatty acid chains was not random as assessed by Merritt (1966), but rather

that the mechanism followed a preferential pattern resulting in an uneven distribution of the hydrocarbons formed. This work agreed with that of Dubravic and Nawar (1969) on fish oils. Quantitative analysis of the volatiles from the irradiated fish oils demonstrated that the major products of irradiation were the longer chain compounds which were considered by Dubravic and Nawar (1969) to have arisen from the fatty acids near the carboxyl group.

The preceding literature review is a general one concerning several aspects of the irradiation sterilization of meats and poultry. It is part of an accumulation of knowledge which is necessary to assure that a new food processing method will render a food safe and nutritious. The present investigation is concerned with yet another aspect of the irradiation sterilization of meats and poultry, the quality of the fatty acids after irradiation sterilization of a chicken-based product.

MATERIALS AND METHODS

Samples

The chicken-based product used in this study consisted of 87 percent chicken in the form of small chunks and 13 percent corn starch which was added for the purpose of binding. The food was commercially prepared and packaged in 300 x 407 cans which contained 14 ounces of the product. The containers were vacuum packed under 18 to 20 inches of reduced Hg pressure. The samples were purchased locally with care so that the cans all contained the same manufacturer's designation to ensure that they were processed in the same batch. The guaranteed proximate analysis on the food as found on the can was as follows:

Crude Protein	11.0% (min.)
Crude Fat	8.0% (min.)
Crude Fiber	1.5% (max.)
Ash	3.5% (max.)
Moisture	74.0% (max.)

The samples irradiated were placed inside diving bells and lowered to the bottom of a 20 ft. well filled with water and allowed to remain in close proximity to the gamma radiation source for predetermined lengths of time in order to accomplish the desired dosage.

Dosimetry

Samples of the chicken product were irradiated in the irradiators located in the Nuclear Science Center at Louisiana State

University. The gamma radiation sources used were a 4419 curie Cobalt-60 irradiator and a 19,850 curie Cobalt-60 irradiator.

The Fricke Dosimetry Method was used for determining the amount of radiation which would be absorbed from the Cobalt-60 sources (Weiss, 1952). The dose rate was measured on the basis of the oxidation of a ferrous ammonium sulfate solution and determination of the ferric ion produced spectro-photometrically. Samples of dosimeter solutions were placed in the radiation field for precisely measured lengths of time. After removal of the specimens from the radiation field, their optical densities were determined immediately in a Beckman type DB spectrophotometer at the wave length 305 milli-microns. A portion of the unirradiated ferrous solution was used as a blank in the spectrophotometer. From the optical density of the irradiated solutions, the radiation dosage was determined from a calibration curve and the following empirical formula:

$$D = 2.94 \times 10^{4} \quad 1 - 0.007 (t-20) \quad (A/T)$$

where

D : Dose rate

t : Ambient room temperature

A : Absorbancy

T : Time of radiation, min.

Figure 1 shows absorbancy vs. time curves for the irradiation of the Fricke dosimetry solutions for three time periods: 5 minutes, 10 minutes and 15 minutes of irradiation. The five different curves in Figure 1 represent five different positions in which

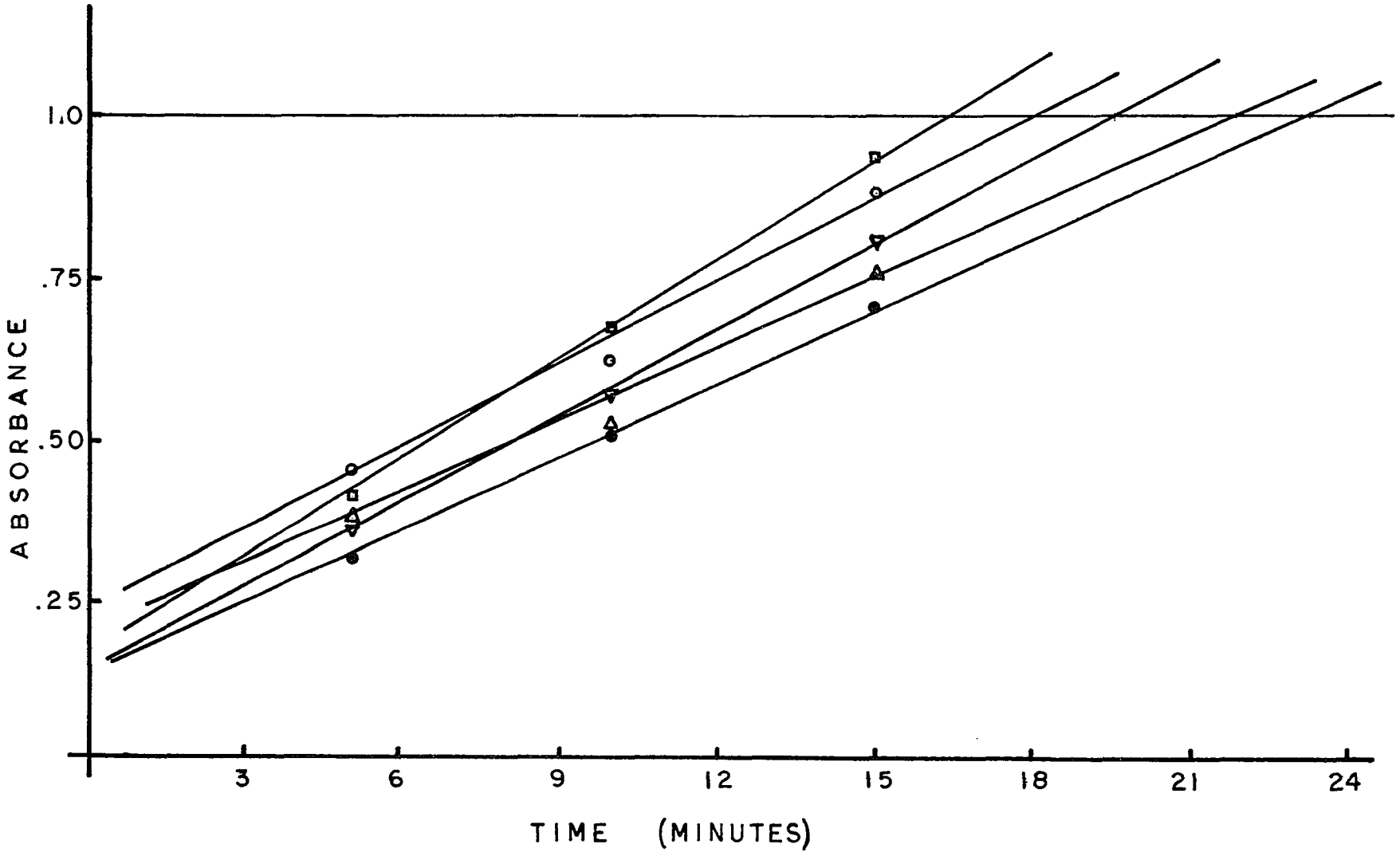


Figure 1. Relationship of absorbance vs. time for five different positions in the irradiation chamber.

the dosimetry solutions were placed in the diving bell used to house the food which was irradiated. It is seen in the figure that the absorbancy is linearly proportional to the time of irradiation of the solution.

The temperature of irradiation, t, the absorbancy, A, and the time of irradiation, T, necessary to give an absorbancy of 1.0 were the necessary factors inserted into the empirical formula of the Fricke dosemetry method. The length of time required to give an absorbancy of 1.0 was obtained by extrapolation of the absorbancy vs. time curve to the 1.0 absorbancy line (Figure 1). By inserting the extrapolated values for the time and using the absorbancy of 1.0 and an ambient temperature of 25 C in the formule, five different dose rates for the five different positions of the Fricke dosimetry solutions were obtained. The mean value of the five determinations was calculated to be 87,258 rads/hour. The time required to obtain a radiation dosage of 4.5 million rads (Mrad) was determined by dividing the dosage required by the dose rate. The time required in the 4419 curie irradiator was 52 hours, and the time required in the 19,850 curie irradiator was 6.0 hours (Lambremont, 1969).

Extraction of Lipids

In order to obtain a sample which would be representative of the whole can of food, the total contents of each can of food of both non-irradiated and irradiated products were each placed in a blender and mixed for one minute. The samples in the blender jars were

allowed to cool in a refrigerator in order to solidify the contents for weighing purposes. The extraction procedure was a modification of the determination which was developed by Folch et al. (1957). The The basic principle was a cold chloroform-methanol extraction. Ten gram samples of the contents of the blender jars were weighed out in Omni Mixer cups and blended with 150 milliliters of cold chloroform-methanol in a 2:1 volume/volume ratio for three minutes. The slurry was filtered into 500 ml filtering flasks by suction using a No. 2 Buchner funnel and two sheets of Whatman No. 1 filter paper. About 30 ml of the cold chloroform-methanol mixture was then used to rinse the mixer cup and the residue. The filtrate was transferred to a 500 ml separatory funnel with another 20 ml chloroform-methanol rinse of the filtering flask. For separating the soluble proteins, 40 ml of cold 0.003 N $MgCl_2$ solution was added to the separatory funnel and shaken vigorously for one minute. The separatory funnel and contents were allowed to stand overnight in a refrigerator at $4^{o}C$ in order to break the water and organic solvent phases. After about 10 hours the lower phase was drained into a 500 ml glass-stoppered Erlenmeyer flask, and dried with granular anhydrous magnesium sulfate. The mixture was then filtered into a 500 ml filtering flask by suction, the Erlenmeyer flask rinsed with 20 ml chloroform and this rinse was then added to the filtering flask. The filtrate was transferred into a 250 ml volumetric flask and diluted to volume (250 ml) with chloroform.

Determination of Total Lipids

Beakers of 100 ml capacity were dried at 195°C overnight and cooled in a desiccator for 30 minutes. The beakers were weighed on an analytical balance and the weights were recorded. Into each beaker 50 ml of the chloroform-methanol extract was transferred. The beakers were placed on a steam bath and the extract was evaporated to a constant weight. The beakers were weighted again (this time with the fat included) on an analytical balance and the weights recorded. The weight of the total lipid was determined according to the following calculation:

Weight of beaker and lipids (g) - weight of beaker (g) =
weight of total lipids (g)/50 ml of the extract or
2 g of chicken product.

Thin-layer Chromatography

Glass plates (20 cm^2) used in thin-layer chromatographic procedures were thoroughly cleansed with detergent and rinsed first with tap water and then with distilled water. Immediately before applying the stationary phase, the plates were scrubbed with methanol. For preparation of plates, 50 g of silica gel G were mixed with 60 ml of distilled water in a Servall Omni-Mixer for 15 sec. A 0.50 millimeter layer of the slurry was spread over the plates with the aid of an adjustable applicator. The plates were air-dried for approximately 30 minutes and activated in a drying-oven at 120°C. The thin-layer plates were stored in a dessicator made especially for this purpose.

After cooling to ambient temperature, a vertical line was drawn on each plate one inch from the edge. A quantitative standard mixture containing phospholipid, cholesterol, stearic acid, tripalmitate and cholesterol-palmitate in chloroform was spotted 2 cm from the bottom of the plate in the area between the line and the edge of the plate. A concentrated solution of lipid sample containing 100 mg lipid/ml of solvent was applied to the remainder of each plate. Fifteen spots each containing 10μl were applied to the plate with a 10μl syringe.

Developing tanks were prepared by lining one side with a sheet of filter paper and adding 250 ml of an 84/15/1 (v/v/v) mixture of petroleum ether, diethyl ether and 35N formic acid, respectively. After allowing the solvents to equilibrate for 30 min., the thin-layer plates containing the lipids were placed in the tanks and developed until the solvent fronts reached 1 cm from the top. The plates were dried by flushing with a stream of nitrogen gas for several minutes. A 0.2 percent solution of 2', 7' dichlorofluorescein in 95 percent ethanol was sprayed evenly over the entire surface of the plates which were subsequently observed under an ultra-violet light source. The lipid fractions were located, marked, and identified by comparison to the spots which had been identified in the standard mixture by R_f values.

Esterification Procedure

The identified lipid fractions on the thin-layer plates were scraped off with a microscopic slide into 25 ml volumetric flasks for the esterification procedure. The transmethylation procedure of Metcalfe et al. (1966) was used for esterification of fatty acids for gas-liquid chromatography. Boiling chips were added to prevent bumping in the volumetric flasks containing the lipid-silica gel scrapings. Four milliliters of 0.5N methanolic sodium hydroxide was added to the mixture for saponification of the lipids. The mixture was then heated to boiling on a steam bath for about 5 minutes. A constant stream of nitrogen gas flowed over the boiling mixture to prevent oxidation of the lipids. Transmethylation resulted when five ml of boron-trifluoride-methanol reagent (BF_3-methanol, Supelco, Inc., Bellefonte, Pa. 16823) was added to the flask and the mixture boiled for two minutes. After cooling the mixture, two ml of nanograde hexane were added to the flask in order to transfer the methyl esters to this solvent for gas-liquid chromatography. Sufficient quantities of a saturated sodium chloride solution were added to the flask to float the fatty acid methyl esters up into the narrow neck of the flask where they were withdrawn by means of a Pasteur pipette.

Gas-Liquid Chromatography

Fatty acid analysis was conducted in a Micro-Tec GC 2000-R gas chromatograph equipped with dual columns and flame-ionization detectors. The operational conditions employed for the analysis is shown in Table 3.

Table 3. Gas chromatograph operational conditions

Column substrate	:	17% diethylene glycol succinate
Colume support	:	chromoport, 80 - 100 mesh
Column length, shape	:	6 feet, coiled helix
Column diameter, inside	:	1/4 inch, stainless steel
Carrier gas	:	nitrogen
Carrier gas pressure	:	40 psig
Carrier gas flow rate	:	80 ml per minute
Operating temperature column	:	190C
detector	:	275C
inlet	:	275C
Output polarity	:	negative
Output attenuator	:	8-32
Input attenuator	:	10^2
Chart speed	:	1 inch per minute
Detector	:	dual hydrogen flame
Recorded	:	Honeywell (Brown Electronik)

Before samples were injected into the gas chromatograph, the columns were conditioned overnight. This was accomplished by programming the thermostat to raise the temperature of the column oven at a rate of 2°C per minute from an initial 50°C to 210°C. The final temperature was dropped to 190°C (operational conditions in this study). The carrier gas was allowed to flow at a rate of 25 ml per minute for the conditioning phase.

Each fatty acid methyl ester sample formed in the transmethylation procedure was concentrated to about 0.2 ml before a microliter portion was injected into the gas chromatograph.

Fatty acids were identified by comparison of observed retention values to those of the fatty acids in the qualitative standards. The standard solution was composed of methyl esters of a homologous series of saturated fatty acids from C_6 to C_{22} and $C_{11:}$, $C_{18:}$ and $C_{18=}$. The standard solution was chromatographed to obtain retention times for comparison with the unknowns.

The areas of the fatty acid peaks were determined with the aid of a planimeter (model 39231 compensating polar planimeter, Gelman Instrument Co., Ann Arbor, Michigan).

RESULTS AND DISCUSSION

Total Lipids

Determination of the total lipids content of 10 gram samples of the non-irradiated and the irradiated chicken product revealed little difference between the total lipid content of the two types of product. The mean values of the total lipid determinations on five samples of both the non-irradiated controls and the irradiated samples were 89.4±5.8 milligrams of fat/gram of non-irradiated product and 94.5±8.2 milligrams of fat/gram of irradiated chicken product.

Classes of Lipids

The classes of lipids found in both the non-irradiated and irradiated samples of the chicken product were phospholipids, sterols, free fatty acids, triglycerides and sterol esters. These classes were tentatively identified chromatographically on thin-layer plates by comparing with standards containing phospholipid, cholesterol, stearic acid, tri-palmitate, and cholesterol-palmitate. It was obvious that the triglyceride fraction constituted the predominant class. This was ascertained by observation of the relative amounts of methyl esters of the various classes required to produce a suitable gas-liquid chromatogram and upon visual observation of developed thin-layer plates under ultra-violet light.

All of the lipid fractions of both the non-irradiated and irradiated samples separated well in the thin-layer chromatographic procedure except the sterol-ester fraction. This fraction did not separate fully from the solvent front which may have been the cause of the variability of the gas-liquid chromatographic results for this fraction. Hydrocarbons migrate with the solvent front in the procedure employed in the present study and this may account for the contamination of the sterol-ester fraction.

Fatty Acids of the Chicken Product

The fatty acid profiles of the total lipid extract of samples of the non-irradiated and irradiated chicken product were determined for two irradiation dose rates. In Table 4 are shown the percentages of fatty acids of the total lipid extract of samples irradiated in the 4410 curie Cobalt-60 irradiator for 52 hours which accomplished a dose of 4.5 Mrad. Percentages of the fatty acids of the total lipid extract of non-irradiated samples from the same original batch but in different cans of chicken product are given in Table 5. Percentages of fatty acids in the chicken product irradiated for 52 hours were similar to those obtained when the samples were irradiated for 6 hours in the 19,850 curie irradiator (Table 6). The absence of the C_8 fatty acid, caprylic, in the samples irradiated in the 4419 curie emitter was noted in Table 4, and this loss of the shorter chain fatty acid could have been due to volatilization. The experiements using the

Table 4. Relative percent fatty acid composition of the total lipid extract of the chicken product irradiated in the Cobalt-60 source for 52 hours (4.5 Mrad)

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$ [a]
C_{10}	0.1	0.5	1.4	0.67±1.66
C_{11-} [b]	0.6	0.5	0.6	0.57±0.15
C_{14}	1.3	1.8	1.7	1.67±0.67
C_{14-}	0.8	0.8	0.7	0.77±0.15
C_{16}	19.8	19.7	23.3	20.27±5.09
C_{16-}	6.5	7.0	6.1	6.51±1.12
C_{18}	5.7	5.1	6.4	5.73±1.61
C_{18-}	41.0	40.2	39.6	40.40±2.12
$C_{18=}$	19.5	19.7	16.6	18.57±4.45
C_{20}	0.6	3.1	0.1	1.27±3.99

[a] Mean ± standard error

[b] Dash denotes the number of double bonds

Table 5. Relative percent fatty acid composition of the total lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$ [a]
C_8	4.2	3.8	3.97	3.97±0.52
C_{10}	1.1	1.7	0.2	1.00±1.88
C_{11-} [b]	1.1	0.1	0.2	0.47±1.36
C_{14}	2.2	2.1	0.97	1.76±1.68
C_{14-}	2.0	0.9	1.56	1.49±1.37
C_{16}	20.5	19.6	18.7	19.65±2.16
C_{16-}	5.8	6.7	6.00	6.18±1.14
C_{18}	6.6	6.0	6.45	6.38±0.77
C_{18-}	37.9	37.0	40.6	38.51±4.04
$C_{18=}$	18.0	19.6	21.1	19.56±3.85
C_{20}	0.2	1.9	0.2	0.76±2.44

[a] Mean ± standard error

[b] Dash denotes the number of double bonds

Table 6. Relative percent fatty acid composition of the total lipid extract of the chicken product irradiated in the Cobalt-60 source for 6 hours (4.5 Mrad).

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$ [a]
C_8	1.85	1.02	0.99	1.29±1.21
C_{10}	0.92	0.68	0.66	0.75±0.36
C_{11-} [b]	1.11	0.85	0.83	0.93±0.39
C_{14}	2.03	1.36	1.82	1.73±0.85
C_{14-}	0.74	0.51	0.66	0.63±0.29
$C_{?}$ [c]	0.92	0.51	0.50	0.64±0.60
C_{16}	20.33	20.41	20.53	20.42±0.29
C_{16-}	7.21	6.46	6.62	6.76±0.99
C_{18}	6.10	6.29	5.96	6.11±0.42
C_{18-}	34.57	36.56	36.42	35.85±2.75
$C_{18=}$	23.29	23.81	24.00	23.70±0.91
C_{20}	0.92	1.53	0.99	1.14±0.83

[a] Mean ± standard error

[b] Dash denotes the number of double bonds

[c] ? indicates an unidentified fatty acid

4419 curie irradiator were preliminary and were accomplished while the apparatus necessary to use the 19,850 curie source was being readied. The percentages of the major fatty acids detected in the non-irradiated samples of the chicken product (Table 7) were similar to those obtained by Bears (1962) as shown in Table 8.

Gas chromatograms of the fatty acid methyl esters of non-irradiated and irradiated samples of the chicken product are reproduced in Figures 2 through 7. Figures 2, 3, and 4 are the gas chromatograms of the fatty acids of samples of the non-irradiated chicken products from 3 different cans and Figures 5, 6, and 7 are those of the irradiated samples from 3 different cans of the product. The fatty acid profiles of the non-irradiated and the irradiated samples are almost identical. Before the fatty acid methyl esters were injected into the gas chromatograph, each sample was concentrated to 0.3 ml by evaporation of the solvent under a nitrogen atmosphere. One-half microliter of each sample was injected into the gas chromatograph. This method proved to be a good procedure for standardizing the concentrations of fatty acid methyl ester solutions for the gas chromatographic analysis. The fatty acid profiles obtained by this procedure were remarkably similar even though each of these chromotograms represented a sample of the chicken product from a separate can.

Table 7. Relative percent fatty acid composition of the total lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$[a]
C_8	1.77	1.67	2.24	1.89±0.76
C_{10}	0.53	1.30	0.93	0.92±0.96
C_{11}-[b]	0.88	1.49	1.31	1.23±0.78
C_{14}	1.77	1.86	1.87	1.83±0.13
C_{14}-	0.53	0.56	0.75	0.61±0.29
$C_?$[c]	0.35	0.37	0.56	0.43±0.29
C_{16}	21.09	20.45	19.59	20.38±1.87
C_{16}-	6.38	6.13	6.53	6.31±0.51
C_{18}	6.21	6.88	6.53	6.57±0.82
C_{18}-	35.46	34.20	34.89	34.85±1.56
C_{18}=	24.29	23.23	23.88	23.80±1.33
C_{20}	0.71	1.86	0.93	1.16±1.52

[a]Mean ± standard error

[b]Dash denotes the number of double bonds

[c]? indicates an unidentified fatty acid

Table 8. A comparison of the percentages of the major fatty acids of chicken found by Beare (1962) and those obtained in the present study.

Fatty Acid Chain Length	Percent of Fatty Acid	
	Beare (1962)	Cook (1969)
C_{16}	24.5	20.4
C_{16-}[a]	3.2	6.3
C_{18}	9.6	6.6
C_{18-}	39.1	34.9
$C_{18=}$	20.8	23.8

[a]Dash denotes the number of double bonds

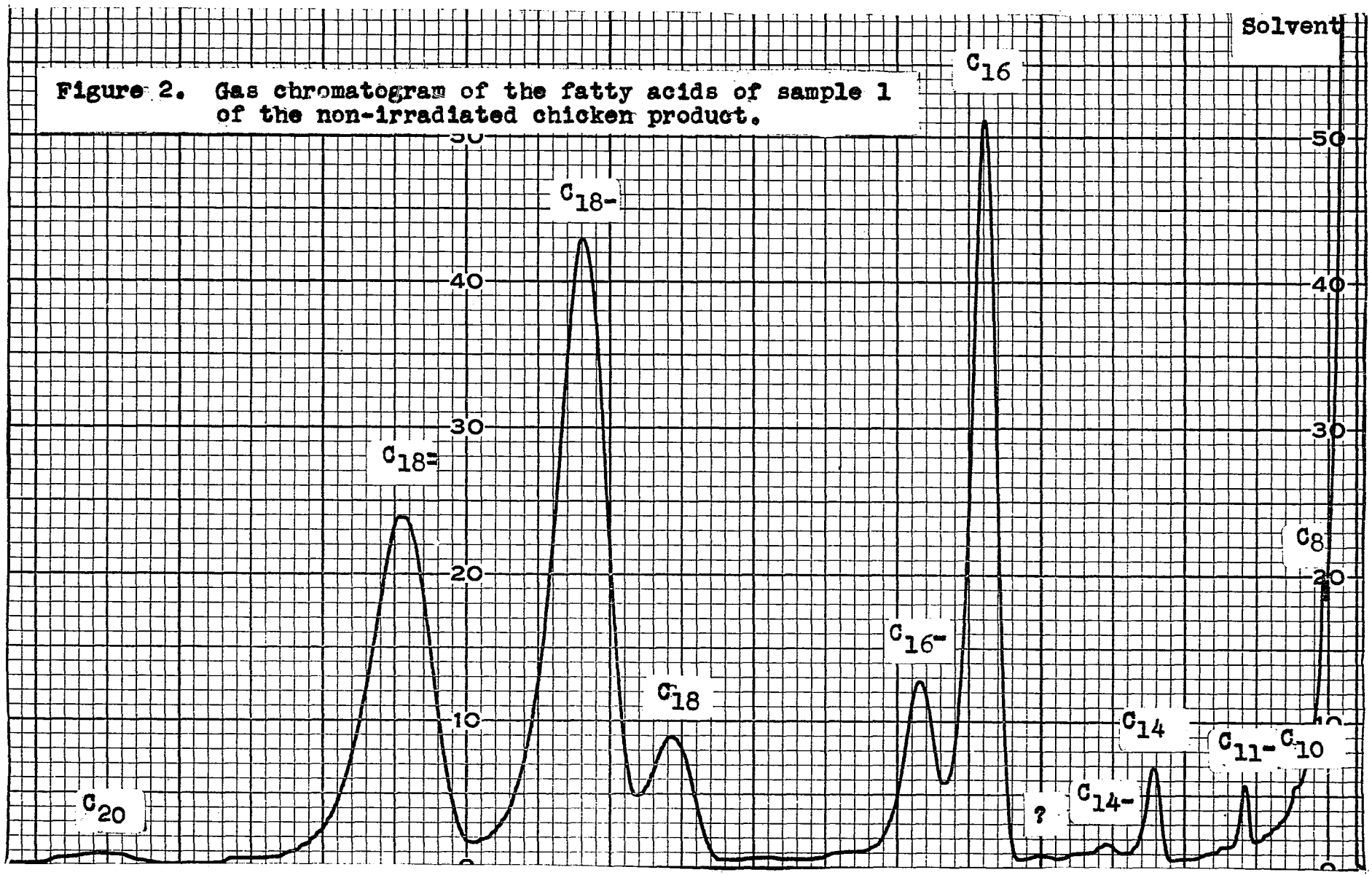


Figure 2. Gas chromatogram of the fatty acids of sample 1 of the non-irradiated chicken product.

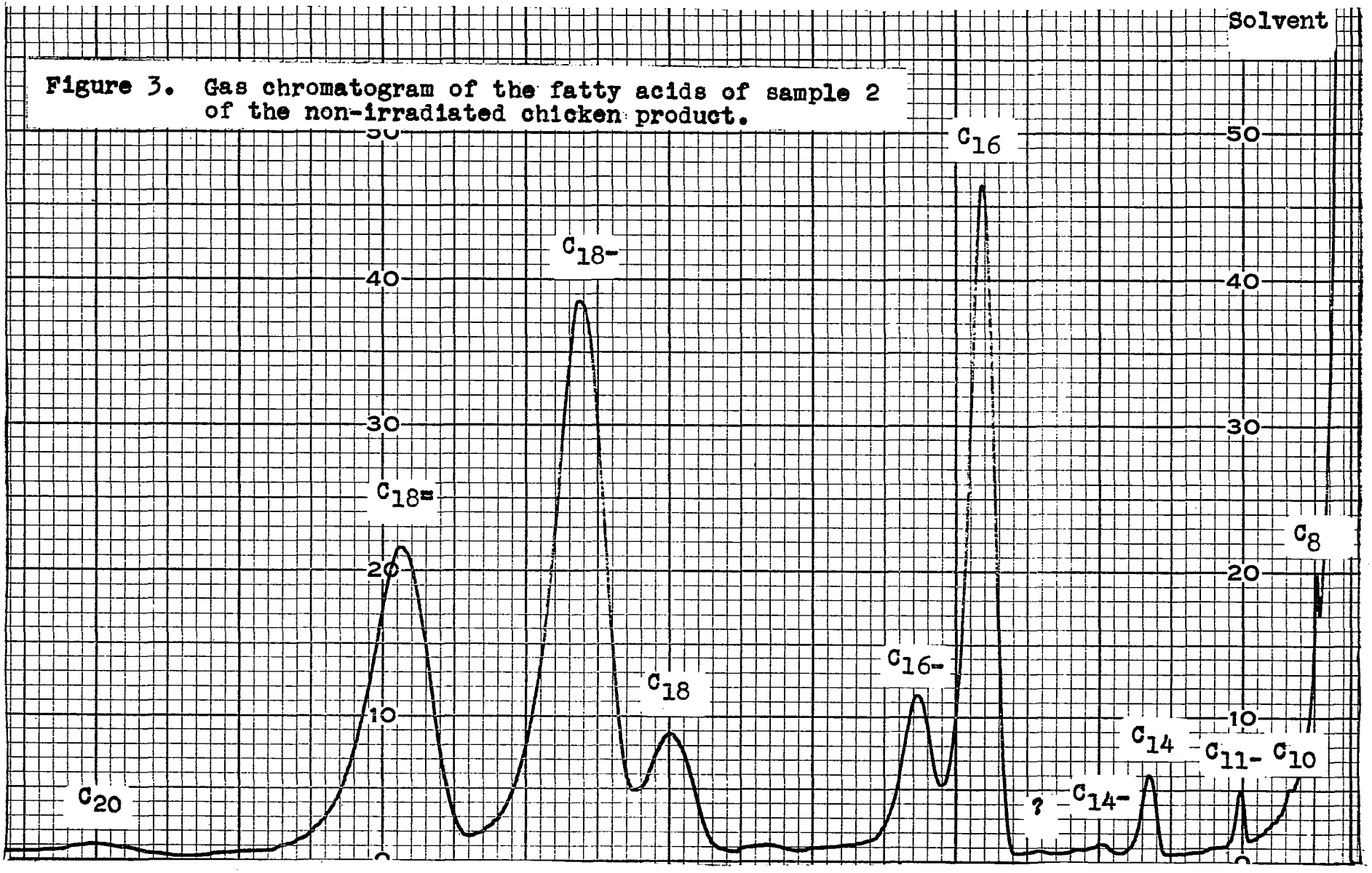


Figure 3. Gas chromatogram of the fatty acids of sample 2 of the non-irradiated chicken product.

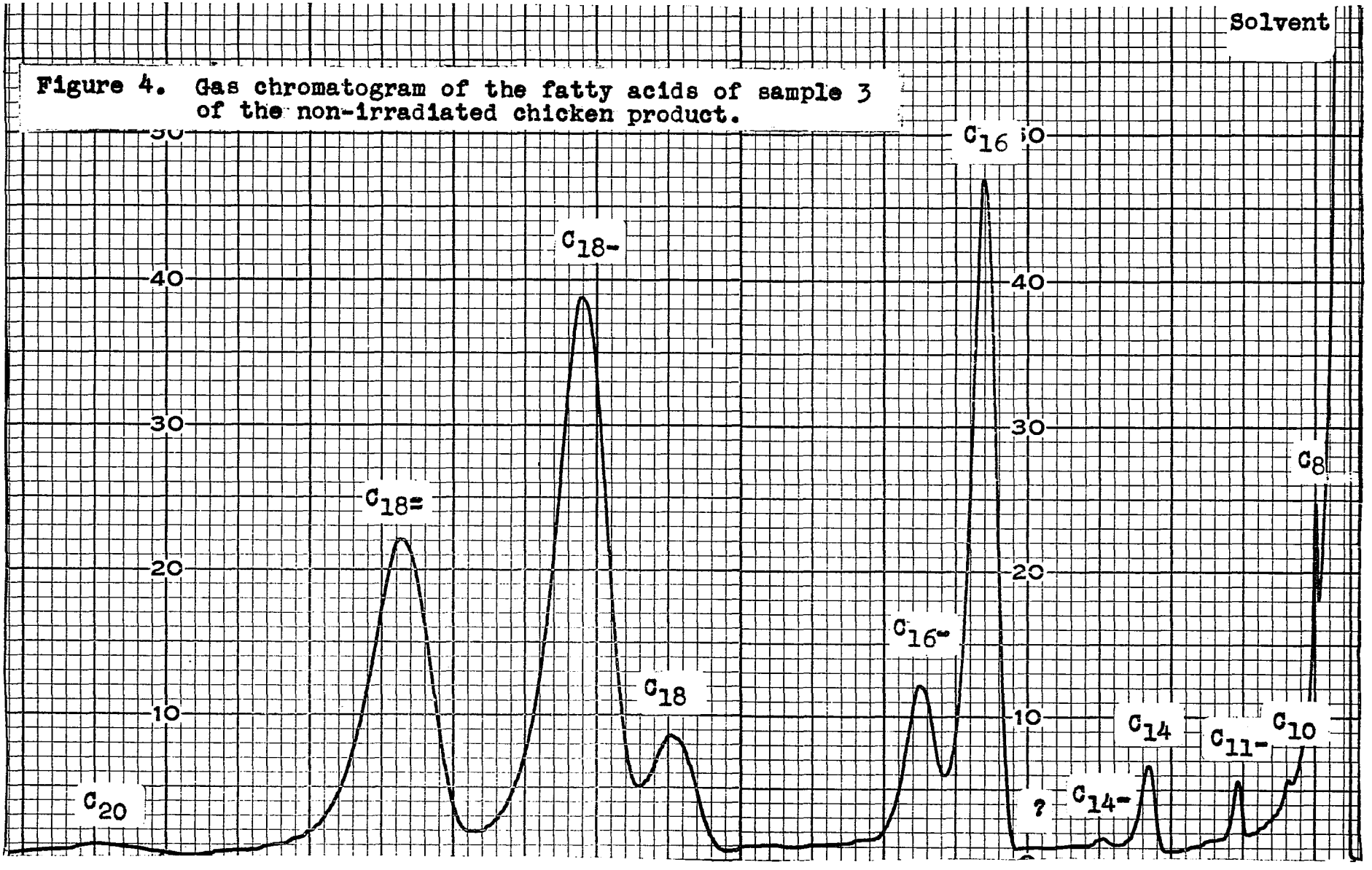


Figure 4. Gas chromatogram of the fatty acids of sample 3 of the non-irradiated chicken product.

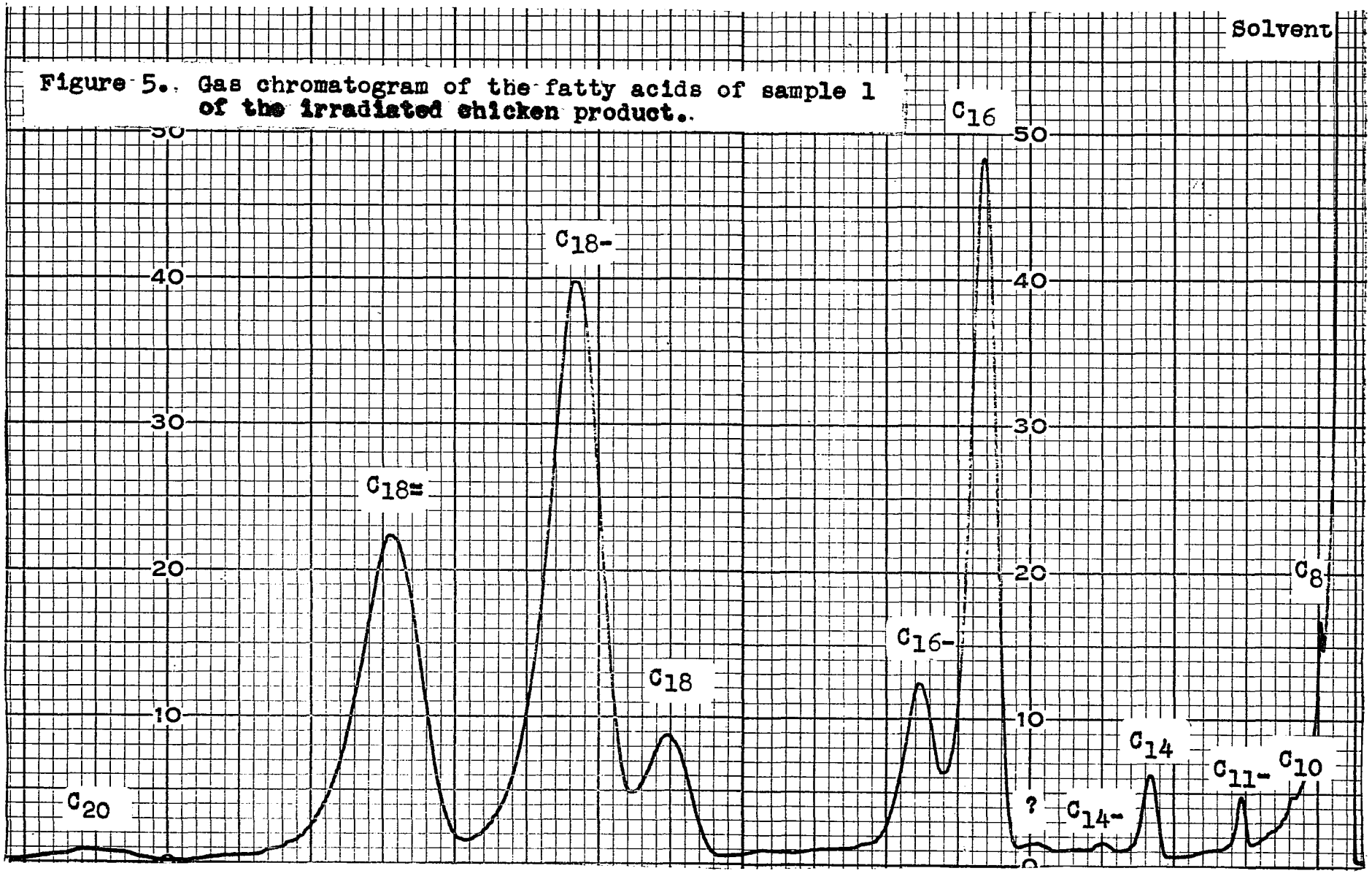


Figure 5.. Gas chromatogram of the fatty acids of sample 1 of the irradiated chicken product..

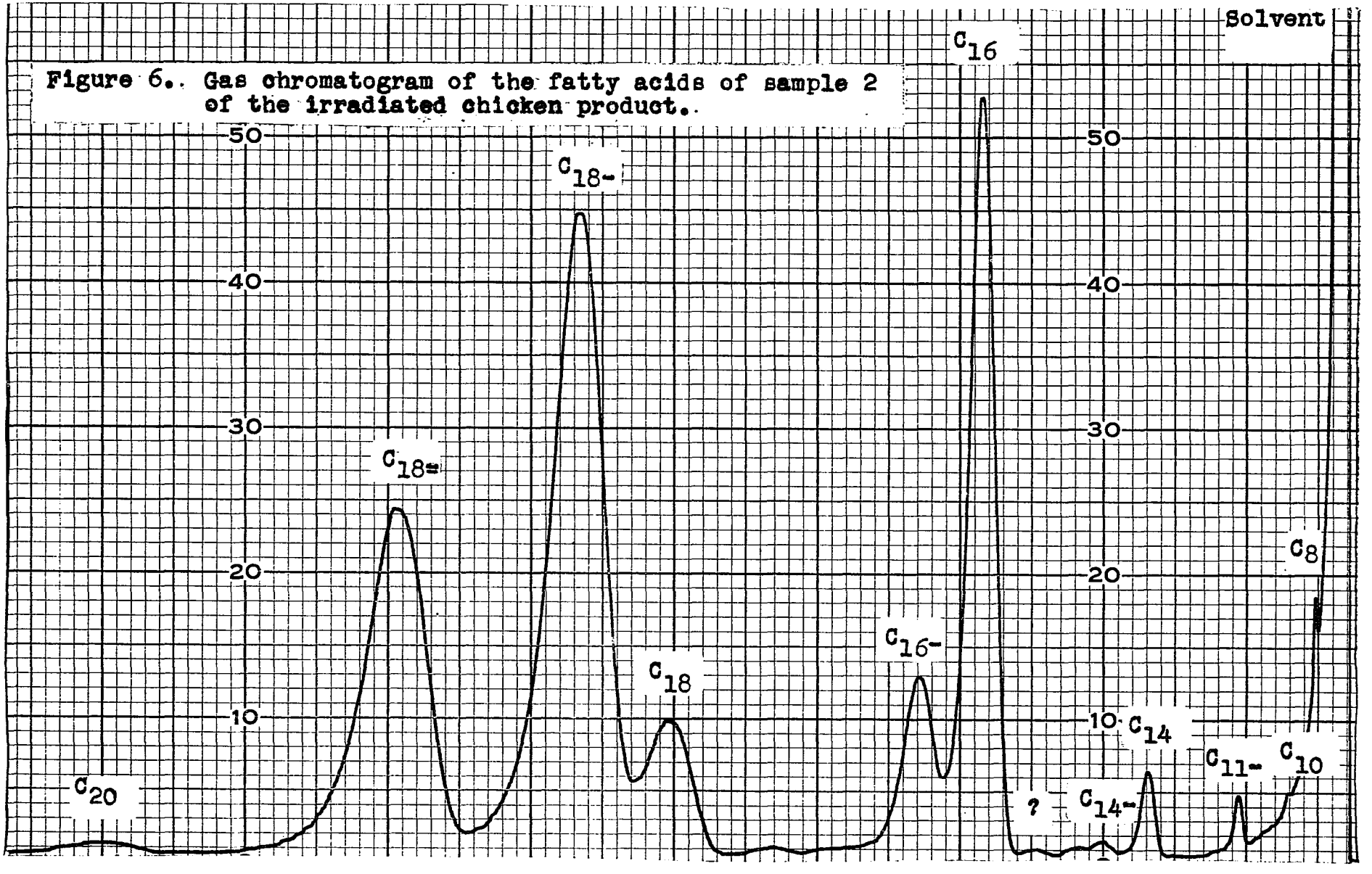


Figure 6.. Gas chromatogram of the fatty acids of sample 2 of the irradiated chicken product..

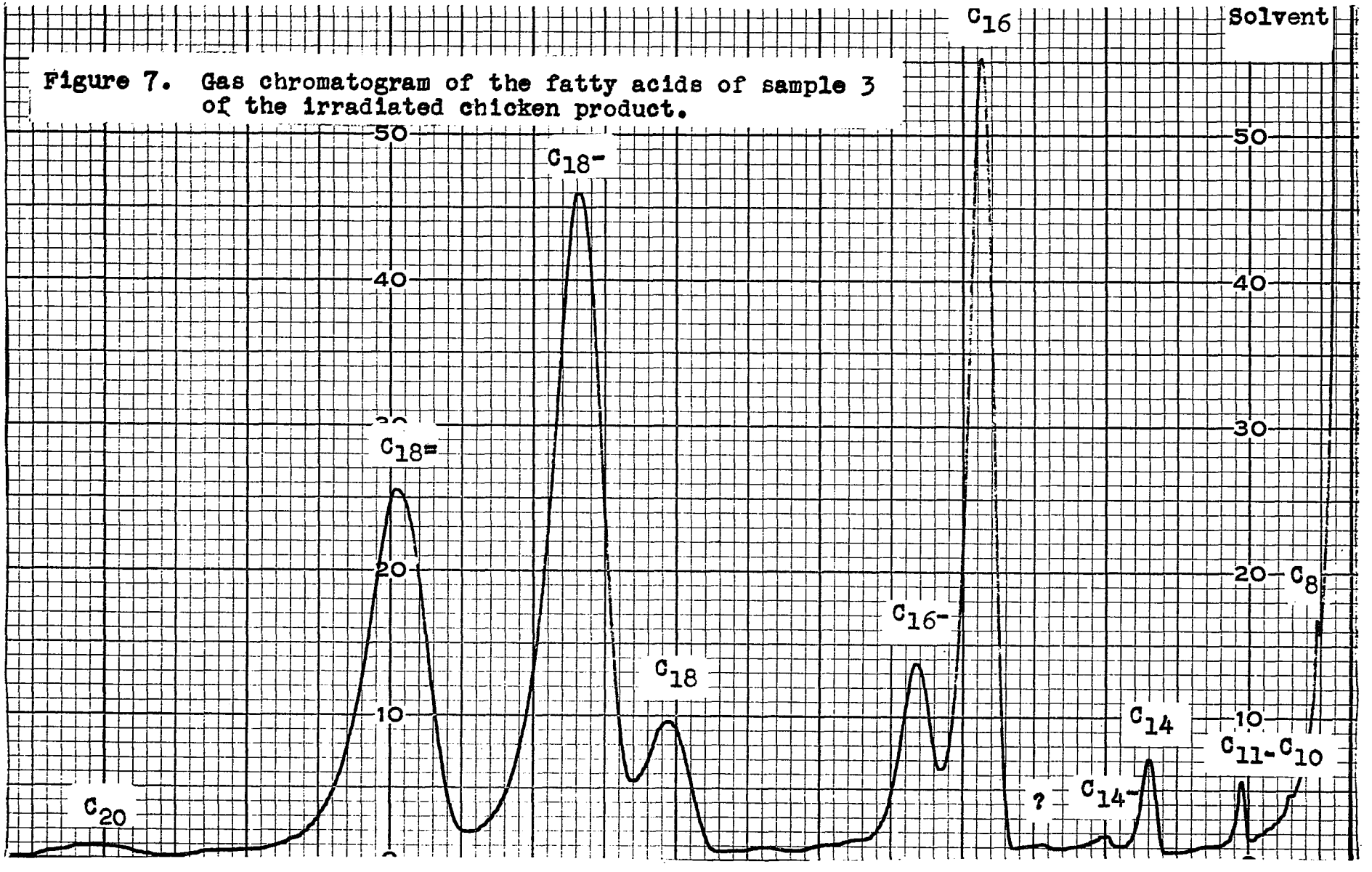


Figure 7. Gas chromatogram of the fatty acids of sample 3 of the irradiated chicken product.

The percentage composition of the various fatty acids detected in the total lipid extracts of the non-irradiated and the irradiated samples of the chicken product are listed in Tables 7 and 8. These tables also contain the calculated means and the standard error of deviation of the samples similarly processed. Diagramatic representations of the fatty acid percentages of the total lipid extract of non-irradiated and irradiated samples of the chicken product are presented in Figure 8. In this figure, the vertical line inside each block represents the standard error of the percent in the fatty acid detected in three samples of both non-irradiated and irradiated products. The paired blocks represent the percent of fatty acid detected in the non-irradiated samples and the unshaded area of the pair is the percent fatty acid of the irradiated sample. The differences in the percent fatty acids of the pairs vary from one fatty acid to another, although percentages of the pairs fall within standard error range of one another. A slightly higher percent of caprylic acid (C_8), capric acid (C_{10}), undecylenic (C_{11-}), myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}), linoleic ($C_{18=}$), and arachidic acid (C_{20}) were found in the non-irradiated samples. Tetradecenoic acid (C_{14-}), an unknown fatty acid, palmitoleic acid (C_{16-}),and oleic acid (C_{18-}) were found in higher percentages in the irradiated samples, but the percentages fell within the standard error of deviation of each other. Because such little differences in the fatty acids of the total extracts of samples of the non-irradiated and the irradiated chicken

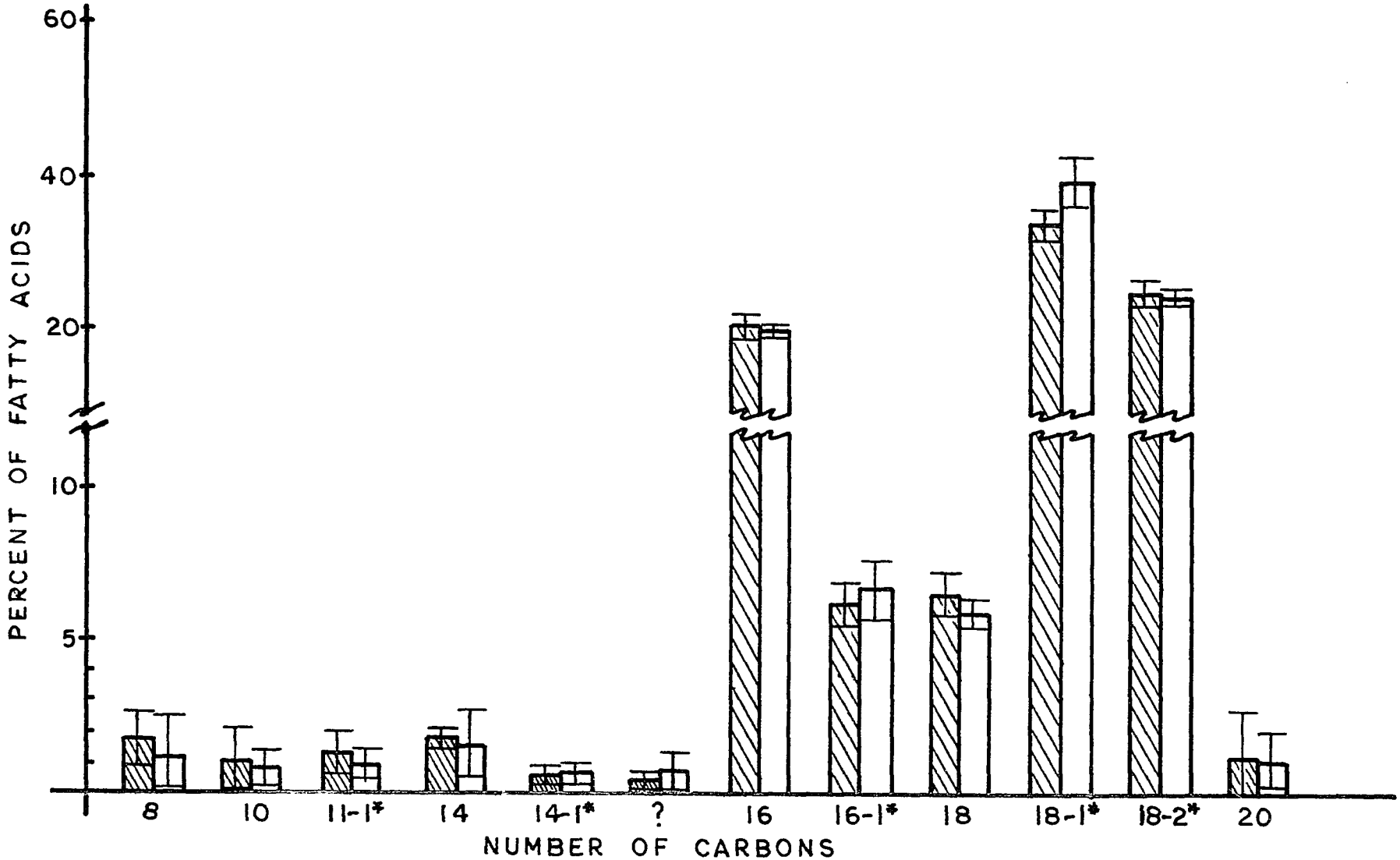


Figure 8. Comparison of the fatty acid percentage of the total lipid extract of non-irradiated and irradiated samples of the chicken product.

non-irradiated irradiated *number of double bonds

product were found, it was decided to study the specific classes of the lipids for possible changes in these fractions.

Fatty Acids of the Lipid Classes

Results of the fatty acid analysis of the phospholipid, sterol, free fatty acid, triglyceride and sterol-ester fractions of the lipid extracts of the samples of non-irradiated and irradiated chicken products are given in Tables 9 through 18. The means and standard error of deviation were calculated on three samples of each of the fractions' fatty acids except the sterol-ester fraction. The results of the fatty acid analysis of the sterol-ester fraction were not consistent, indicating contamination and resulting in poor resolution of the fatty acids of this fraction. The standard error was high among samples of the same fraction except for the triglyceride fraction which gave results similar to those of the fatty acid percentages of the total lipid extracts. The differences in the percent of fatty acids of the non-irradiated and the irradiated samples of the same class were not consistent in the composition of saturated and unsaturated fatty acids. To further explain this result, in the phospholipid fraction fatty acid analysis (Tables 9 and 10) the undecylenic acid (C_{11-}) palmitoleic acid (C_{16-}), and linoleic ($C_{18=}$) were detected in slightly smaller concentrations in the irradiated samples whereas the other unsaturated fatty acids detected were found in comparatively larger concentrations in the irradiated samples. In the sterol fraction (Tables 11 and 12), only a trace of capric acid was found in the

Table 9. Relative percent fatty acid composition of the phospholipid fraction of the lipid extract of the irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$ [a]
C_{10}	tr.[b]	-[c]	-	
C_{11}-[d]	3.2	1.4	2.4	2.33±2.24
C_{14}	4.2	4.2	3.3	3.90±1.29
C_{14}-	3.9	8.5	3.1	5.16±7.24
C_{16}	20.2	20.70	21.50	20.80±1.63
C_{16}-	4.2	5.6	4.4	4.73±1.88
C?[e]	0.9	5.3	1.2	2.43±6.12
C_{18}	13.0	14.2	11.4	12.87±3.49
C_{18}-	29.0	22.7	29.3	27.00±9.26
C_{18}=	20.9	17.0	19.7	19.20±4.96
C_{20}	-	-	0.6	

[a] Mean ± standard error

[b] Trace amounts

[c] - not detected

[d] Dash denoted the number of double bonds

[e] ? indicates an unidentified fatty acid

Table 10. Relative percent fatty acid composition of the phospholipid fraction of the lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05}S_{\bar{X}}$ [a]
C_{10}	-[b]	-	tr.[c]	
C_{11-}[d]	3.6	7.4	3.8	4.93±5.31
C_{14}	3.6	3.7	3.9	3.73±0.38
C_{14-}	3.8	6.7	4.1	4.87±3.96
C_{16}	21.1	25.3	19.9	22.10±7.04
C_{16-}	3.4	6.7	4.6	4.90±4.15
$C_{?}$[e]	0.9	3.7	3.3	2.63±3.76
C_{18}	18.7	16.2	18.4	17.77±3.39
C_{18-}	26.5	18.6	23.5	22.87±8.36
$C_{18=}$	18.0	12.6	17.4	19.33±7.35
C_{20}	-	-	0.8	

[a] Mean ± standard error

[b] - not detected

[c] Trace amounts

[d] Dash denoted the number of double bonds

[e] ? indicates an unidentified fatty acid

Table 11. Relative percent fatty acid composition of the sterol fraction of the lipid extract of the irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$[a]
C_{10}	tr.[b]	tr.	tr.	
C_{11-}[c]	16.4	14.70	12.5	14.53± 4.86
C_{14}	18.6	11.7	9.3	13.20±11.99
C_{14-}	13.9	4.9	tr.	
C_{16}	15.1	19.6	20.8	18.50± 7.46
C_{16-}	5.8	4.9	6.2	5.63± 1.66
C_{18}	5.8	9.8	4.1	6.57± 7.27
C_{18-}	17.4	18.6	27.0	21.00±12.99
$C_{18=}$	5.8	15.6	19.7	13.70±17.74

[a]Mean ± standard error

[b]Trace amounts

[c]Dash denotes the number of double bonds

Table 12. Relative percent fatty acid composition of the sterol fraction of the lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$[a]
C_{10}	14.9	14.2	17.1	15.40± 3.76
C_{11-}[b]	10.0	13.3	9.1	11.57± 4.12
C_{14}	8.9	12.2	11.4	10.80± 4.28
C_{14-}	3.5	4.7	4.5	4.23± 1.60
C_{16}	17.9	19.0	16.0	17.63± 3.77
C_{16-}	15.5	8.5	2.2	8.73±16.52
C_{18}	5.9	4.7	22.8	11.13±25.15
C_{18-}	13.7	13.3	11.4	12.80± 3.05
$C_{18=}$	8.9	9.5	5.1	7.83± 5.92

[a]Mean ± standard error

[b]Dash denotes the number of double bonds

Table 13. Relative percent fatty acid composition of the free fatty acid fraction of the lipid extract of the irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$ [a]
C_{11-} [b]	16.4	9.2	8.9	11.50±10.55
C_{14}	18.8	12.9	12.5	14.40± 8.76
C_{16}	23.8	23.10	23.20	23.03± 0.96
C_{16-}	1.1	8.3	4.4	4.47± 8.95
C_{18}	10.5	9.2	8.9	9.53± 2.11
C_{18-}	17.6	18.5	22.3	19.47± 6.19
$C_{18=}$	11.7	18.5	12.5	14.23± 9.23

[a] Mean ± standard error

[b] Dash denotes the number of double bonds

Table 14. Relative percent fatty acid composition of the free fatty acid fraction of the lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05} S_{\bar{X}}$ [a]
C_{10}	tr.[b]	tr.	tr.	
C_{11-}[c]	13.5	21.6	14.6	16.53±1.92
C_{14}	13.5	20.0	16.5	16.67±8.08
C_{14-}	9.0	tr.	tr.	
C_{16}	24.3	25.0	24.3	24.53±1.01
C_{18}	9.0	8.3	12.6	9.97±5.73
C_{18-}	21.6	16.6	22.3	20.17±7.72
$C_{18=}$	9.0	8.3	9.7	9.00±1.73

[a]Mean ± standard error

[b]Trace amounts

[c]Dash denotes the number of double bonds

Table 15. Relative percent fatty acid composition of the triglyceride fraction of the lipid extract of the irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05}S_{\bar{X}}$ [a]
C_{10}	1.84	tr.[b]	tr.	
C_{11-}[c]	1.28	1.00	0.9	1.06±0.49
C_{14}	2.56	2.00	1.80	2.12±0.97
C_{14-}	1.54	0.5	0.8	0.95±1.33
C_{16}	22.6	21.3	19.6	21.17±3.73
C_{16-}	7.7	6.5	7.5	7.23±1.60
C_{18}	5.65	6.1	6.8	6.18±1.44
C_{18-}	30.8	37.5	36.6	34.93±9.04
$C_{18=}$	23.8	23.4	23.4	23.53±0.58
C_{20}	2.2	1.2	2.1	1.83±1.37

[a] Mean ± standard error

[b] Trace amounts

[c] Dash denotes the number of double bonds

Table 16. Relative percent fatty acid composition of the triglyceride fraction of the lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3	$\bar{X} \pm t_{.05}S_{\bar{X}}$ [a]
C_{10}	tr.[b]	2.3	tr.	
C_{11-}[c]	0.9	1.3	1.2	1.13±0.52
C_{14}	1.8	1.6	2.2	1.87±0.76
C_{14-}	0.9	0.8	1.3	1.00±0.66
C_{16}	19.8	21.1	20.5	20.47±1.62
C_{16-}	7.2	6.7	6.7	6.87±0.72
C_{18}	5.4	5.0	5.3	5.23±0.52
C_{18-}	36.9	35.5	36.2	36.20±1.74
$C_{18=}$	26.4	23.3	24.5	24.70±3.82
C_{20}	0.5	1.0	1.8	1.10±1.63

[a]Mean ± standard error

[b]Trace amounts

[c]Dash denotes the number of double bonds

Table 17. Relative percent fatty acid composition of the sterol-ester fraction of the lipid extract of the irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3
C_{10}	10.9	9.2	24.8
C_{11}	16.3	-[a]	-
C_{11-}[b]	18.1	14.1	33.3
C_{14}	9.4	17.0	tr.[c]
C_{14-}	14.5	-	14.7
C_{16}	15.6	23.4	19.3
C_{16-}	9.0	7.0	-
C_{18}	-	11.3	-
C_{18-}	5.8	17.7	7.7
C_{20}	tr.	-	-

[a]- not detected

[b]Dash denotes the number of double bonds

[c]Trace amounts

Table 18. Relative percent fatty acid composition of the sterol-ester fraction of the lipid extract of the non-irradiated chicken product.

Fatty Acid Chain Length	Sample 1	Sample 2	Sample 3
$C_{?}$[a]	9.2	-[b]	-
C_{10}	15.4	8.7	11.3
C_{11}	-	15.3	25.8
C_{11-}[c]	-	24.1	26.3
C_{12}	-	tr.[d]	tr.
$C_{?}$	14.7	-	-
C_{16}	13.6	13.1	13.1
C_{16-}	-	10.9	4.4
$C_{?}$	7.3	tr.	-
C_{18}	5.1	-	-
C_{18-}	8.1	6.5	6.5
$C_{18=}$	-	6.5	3.4

[a]?unidentified fatty acid

[b]- not detected

[c]Dash denotes the number of double bonds

[d]Trace amounts

irradiated samples but it was one of the major fatty acids found in the non-irradiated samples. Oleic acid was in a larger concentration in irradiated samples than in non-irradiated samples of the sterol class. The amount of stearic acid of the sterol class detected in the irradiated samples was smaller than the stearic acid in the non-irradiated samples. Palmitoleic acid was not detected in the free fatty acid class of the non-irradiated samples but it was found as the smallest percentage of the fatty acids in the free fatty acid class of the irradiated samples (Tables 13 and 14).

The triglyceride class fatty acid profiles were in close agreement to the total lipid extract fatty acid profiles. A diagramatic representation of the fatty acid of non-irradiated and irradiated samples of the triglyceride class is presented in Figure 9. The standard error of deviation among samples of each fatty acid detected in the triglyceride class was small for both the irradiated and non-irradiated samples. The concentrations of undecylenic acid (C_{11-}), tetradecenoic acid (C_{14-}), oleic acid (C_{18-}) and linoleic acid ($C_{18=}$) in the triglyceride fraction were smaller in the irradiated samples than in the non-irradiated samples. The concentration of palmitoleic (C_{16-}) was slightly larger in the triglyceride fraction of the irradiated samples.

From the results of the total lipid extract fatty acid determinations plus determination of the fatty acids in each lipid class, no difference in the quality of the fatty acids between irradiated

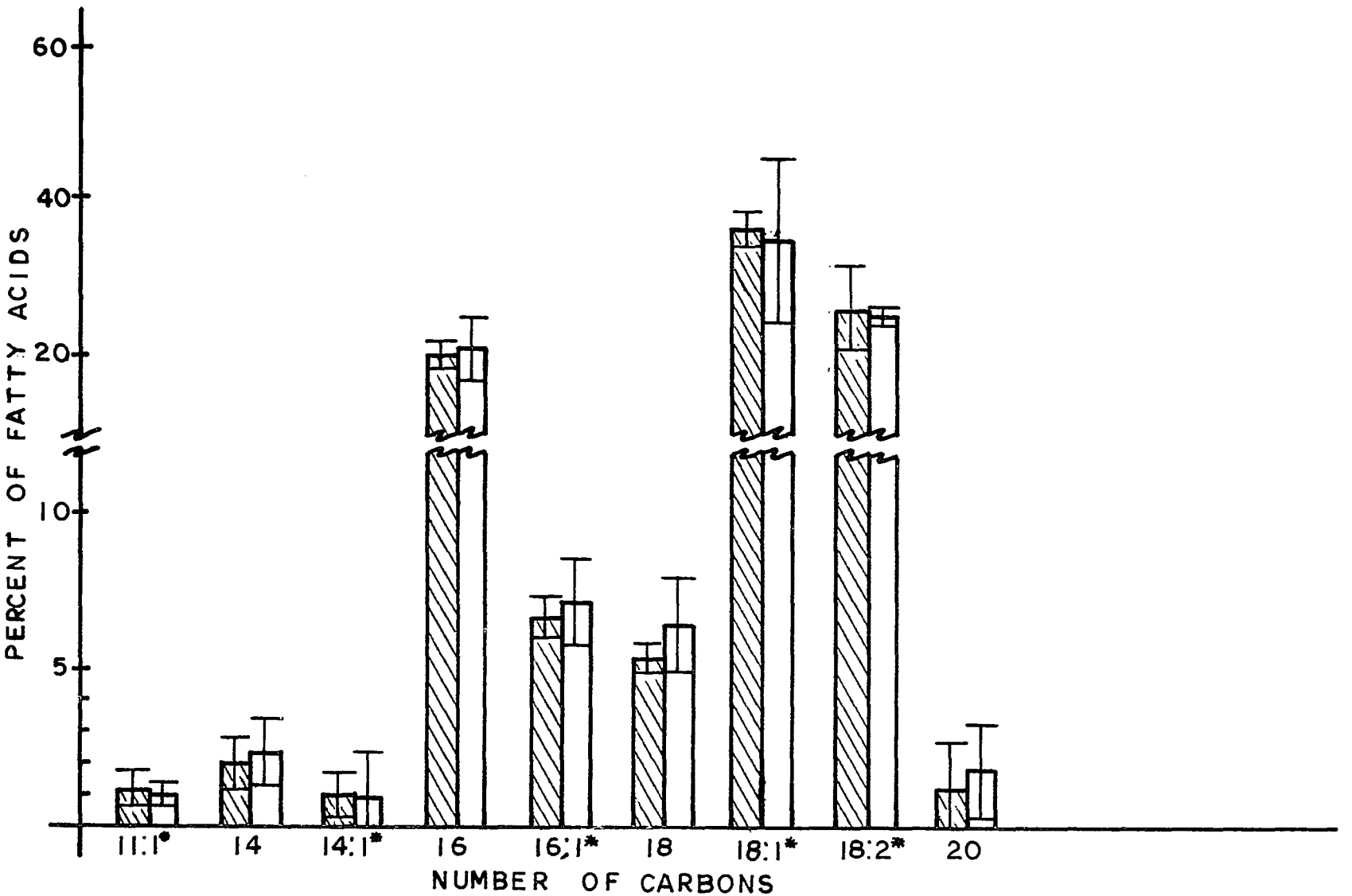


Figure 9. Comparison of the fatty acid percentages of the triglyceride fraction of the lipid extract of non-irradiated and irradiated samples of the chicken product.

non-irradiated irradiated *number of double bonds

and non-irradiated samples of the chicken product. This finding might be due to a masking effect on the fatty acids by the other constituents of the chicken product. The total lipid determinations also demonstrated no differences between non-irradiated and irradiated samples of the chicken product.

The findings of the present investigation will add to the vast knowledge which is being accumulated to support the thesis that radiation sterilization of foodstuffs renders products which are safe and nutritious. The results of this study, the stability of the fatty acids of a chicken product when irradiated at sterilizing doses, are further evidence of the nutritional stability of irradiated products with reference to fatty acids.

Investigations by others indicate that radiation in the sterilizing range has relatively little effect on the digestibility of meats. Long-term studies on the possible toxicity of irradiated foods also indicate no significant effects. These results are based on weight gain of rats and mice which were fed high levels of meat which had been irradiated in their diets. Histological examination of tissues of the experimental animals also revealed no significant differences (Land and Bassler, 1966).

Since minimal odor and flavor changes are observed in poultry products, methods may be devised to prevent or reduce to a minimum the changes that do occur. Absorption with charcoal or masking of these odors and flavors with combinations of spices may be of value

in reducing the odor and flavor changes (Hansen, 1966). Reactions to odors and flavors vary widely among individuals, some indicating a preference for irradiated meat (Heighiman, 1965). The consumer usually does not show significant preferences between non-irradiated meat and meat irradiated in the sterilizing range.

There are certain areas where irradiation sterilization will play an important part in the future. Where the cold chain, that is, keeping food products cold from processor to market, operates ineffectively or not at all, irradiation might well become an important method of preserving food products of all kinds. A likely practical application seems to be the decontamination of imported foods. In defense needs, where convenience and suitability are important, the irradiation of a variety of food products might well solve difficult feeding problems.

SUMMARY

Studies were conducted on the lipids of non-irradiated and irradiated chicken based pet foods. The total lipid extract of each type of product was fractionated employing thin-layer chromatography. Lipid classes, namely phospholipids, sterols, free fatty acids, triglycerides and sterol esters, were demonstrated in the non-irradiated and the irradiated samples of the chicken product. The triglyceride fraction was observed to be the most abundant of the total lipids of both the types of products. The quantities of the lipid classes of both non-irradiated and irradiated chicken product samples were similar.

Fatty acids in the total extract and the lipid fractions of the non-irradiated and the irradiated chicken products were analyzed by gas-liquid chromatography. The spectra of fatty acids tentatively identified in both types of product were caprylic acid (C_8), capric acid (C_{10}), undecylenic acid (C_{11-}), myristic acid (C_{14}), tetradecenoic acid (C_{14-}), palmitic acid (C_{16}), palmitoleic acid (C_{16-}), stearic acid (C_{18}), oleic acid (C_{18-}), linoleic ($C_{18=}$) and arachidic acid (C_{20}). Only one fatty acid was unidentified and it had a retention time between that of tetradecenoic acid and palmitic acid. The fatty acids that consituted a large percentage (80%) of the total composition of both non-irradiated and the irradiated samples' lipid were

identified as palmitic acid, oleic acid and linoleic acid. There were no differences detected between the relative percent fatty acid composition of the non-irradiated and irradiated samples.

The fatty acid composition of the lipid classes were similar. The triglyceride fraction was observed to be the most similar fraction to the total lipids extract in the types and percentages of fatty acids in both the non-irradiated and the irradiated samples of the chicken product. No differences were observed in the relative percent fatty acid composition in the triglyceride of the non-irradiated and irradiated samples.

From the results of the total lipid extract fatty acid determinations plus determination of the fatty acids in each lipid class, no difference in the quality of the fatty acids between irradiated and non-irradiated samples of the chicken product were found. The total lipid determinations also demonstrated no differences between non-irradiated and irradiated samples of the chicken product.

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VITA

William Jackson Cook, Jr. was born on December 18, 1938 in Baton Rouge, Louisiana. In the fall of 1957 he entered Louisiana State University in Baton Rouge where he received the Bachelor of Arts degree in June, 1961.

He entered the Graduate School of Louisiana State University in September of 1961 and was awarded the degree of Master of Science in Microbiology with a minor in Biochemistry in June, 1963. After receiving the M.S. degree, he went to work for the United States Public Health Service Hospital in Carville, Louisiana as a research microbiologist. He married Hortense Estelle Moreau in November of 1963 and they have two children, William III born in September, 1964 and Rachel Elizabeth born in April, 1966.

In September, 1966 he returned to the Graduate School of Louisiana State University. He is presently a candidate for the degree of Doctor of Philosophy in Food Science and Technology with a minor in Microbiology.

EXAMINATION AND THESIS REPORT

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Major Field: Food Science and Technology

Title of Thesis: Fatty Acids in an Irradiated Chicken Product

Approved:

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